

Application No. 09/990,087

Amendment dated April 24, 2003

Response to Office Action dated March 24, 2003

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of claims:

1. (Original) A membrane scaffold protein that will, in an aqueous environment, self assemble in the absence of phospholipid or with a phospholipid ora mixture of phospholipids, into a nanoscale particle between about 5 nm and about 500 nm in diameter, wherein said membrane scaffold protein is amphipathic, and wherein said membrane scaffold protein forms at least one alpha helix.
2. (Original) The membrane scaffold protein of claim 1, wherein said membrane scaffold protein assembles with a phospholipid or a mixture of phospholipids into a nanoscale particle of about 5 nm and about 500 nm in diameter, wherein a phospholipid bilayer is formed.
3. (Original) The membrane scaffold protein of claim 2, wherein the phospholipid bilayer is discoidal.
4. (Original) The membrane scaffold protein of claim 1, wherein said membrane scaffold protein selfassembles together with at least one hydrophobic or partially hydrophobic protein to form a nanoscale particle between about 5 nm and 500 nm in diameter, said nanoscale particles comprising the membrane scaffold protein and the at least one hydrophobic or partially hydrophobic protein.
5. (Previously amended) The membrane scaffold protein of claim 1, wherein said membrane scaffold protein self assembles in the absence of phospholipid to form a nanoscale particle between about 5 nm and about 500 nm in diameter.

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6. (Original) The membrane scaffold protein of claim 5, wherein said nanoscale particle is from about 5 to about 100 nm in diameter.
7. (Original) The membrane scaffold protein of claim 6, wherein said nanoscale particle is from about 5 to about 50 nm in diameter.
8. (Previously amended) The membrane scaffold protein of claim 1, wherein said membrane scaffold protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:23, SEQ ID NO:29, SEQ ID NO:43, SEQ ID NO:44 and SEQ ID NO:45.
9. (Previously amended) A nanoscale particle comprising the membrane scaffold protein of claim 1 and at least one hydrophobic or partially hydrophobic protein, and optionally further comprising a phospholipid or a mixture of phospholipids, wherein said nanoscale particle has a diameter between about 5 nm and about 500 nm.
10. (Original) The nanoscale particle of claim 9, wherein the hydrophobic or partially hydrophobic protein is a membrane protein.
11. (Original) The nanoscale particle of claim 10, wherein said membrane protein is a tethered membrane protein.
12. (Original) The nanoscale particle of claim 10, wherein the membrane protein is an embedded membrane protein.
13. (Original) The nanoparticle assembly of claim 10, wherein the membrane protein is an integral membrane protein.

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14. (Original) The nanoscale particle of claim 13, wherein the membrane protein has seven transmembrane segments.
15. (Original) The nanoscale particle of claim 10, wherein said membrane protein is a receptor protein.
16. (Original) The nanoscale particle of claim 10, wherein said membrane protein is a G-protein coupled receptor.
17. (Original) The nanoscale particle of claim 16, wherein said G-protein coupled receptor is a 5-hydroxytryptamine receptor.
18. (Original) The nanoscale particle of claim 8, wherein said membrane scaffold protein is fused genetically with the hydrophobic protein.
19. (Previously amended) The nanoscale particle of claim 9, wherein said membrane scaffold protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:23, SEQ ID NO:29, SEQ ID NO:43, SEQ ID NO:44 and SEQ ID NO:45.
20. (Original) A method for incorporating at least one hydrophobic or partially hydrophobic protein into a nanoscale particle which is stable and soluble in aqueous solutions, said method comprising the step of allowing a membrane scaffold protein and at least one hydrophobic or partially hydrophobic protein to self assemble into nanoscale particles in an aqueous solution, optionally in the presence of at least one phospholipid, whereby nanoscale particles are formed.

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21. (Original) The method of claim 20, wherein said at least one hydrophobic or partially hydrophobic protein is a membrane protein.
 22. (Original) The method of claim 21, wherein said membrane protein is a tethered membrane protein, an embedded membrane protein or an integral membrane protein.
 23. (Original) The method of claim 22, wherein said membrane protein is tissue factor.
 24. (Original) The method of claim 21, wherein said membrane protein is a receptor protein.
 25. (Original) The method of claim 24, wherein said receptor protein is a G-protein coupled receptor.
 26. (Original) The method of claim 25, wherein said G-protein coupled receptor is a 5-hydroxytryptamine receptor.
 27. (Previously amended) The method of claim 20, wherein said membrane scaffold protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:23, SEQ ID NO:29, SEQ ID NO:43, SEQ ID NO:44 and SEQ ID NO:45.
 28. (Previously amended) The method of claim 20, wherein said at least one hydrophobic or partially hydrophobic protein is associated with membranes or membrane fragments.
 29. (Currently amended) A method for identifying an a competitor of binding of a ligand to a receptor protein wherein said receptor protein is incorporated within a nanoscale particle together with a membrane scaffold protein, said method comprising the steps of:

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- (a) contacting a nanoscale particle comprising a membrane scaffold protein and a receptor protein with a detectable ligand to produce nanoscale particle-bound detectable ligand;
 - (b) contacting the nanoscale particle-bound ligand with a test compound;
 - (c) measuring detectable ligand released from the nanoscale particles;
- whereby a competitor of ligand binding is identified when contacting the nanoscale particle-bound ligand results in release of the detectable ligand.

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- 30. (Previously amended) The method of claim 29, wherein said receptor protein is a membrane protein.
 - 31. (Previously amended) The method of claim 30, wherein said receptor protein is a G-protein coupled receptor.
 - 32. (Previously amended) The method of claim 31, wherein said G-protein coupled receptor is a 5-hydroxytryptamine receptor.
 - 33. (Previously amended) The method of claim 29, wherein said membrane scaffold protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:23, SEQ ID NO:29, SEQ ID NO:43, SEQ ID NO:44 and SEQ ID NO:45.
 - 34. (Previously amended) A DNA molecule encoding a membrane scaffold protein, wherein said membrane scaffold protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:23, SEQ ID NO:29, SEQ ID NO:43, SEQ ID NO:44 and SEQ ID NO:45.

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35. (Previously amended) A recombinant host cell comprising the DNA molecule of claim 34.

36. (New) A method of using the DNA molecule of claim 34 to produce a membrane scaffold protein, said method comprising the steps of:

- a) transforming a host cell capable of expressing a membrane scaffold protein, wherein the DNA molecule comprises a promoter functional in said host cell and a portion encoding the membrane scaffold protein, said portion encoding the membrane scaffold protein operably linked to the promoter; and
- b) culturing the transformed host cell under conditions suitable for expression of said membrane scaffold protein coding sequence.

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